

What is claimed is:

1. A method of assaying a sample of blood or blood components for the presence of 25-hydroxy-vitamin D comprising:
 - 5 (a) lowering the pH of the sample to 5.5 or less to dissociate the 25-hydroxy-vitamin D from vitamin D binding proteins; and
 - (b) determining the concentration of 25-hydroxy-vitamin D in the sample,
wherein the vitamin D binding proteins are not removed from the
10 sample.
2. The method of claim 1, wherein the pH of the sample is lowered to 5 or less.
- 15 3. The method of claim 1, wherein the pH of the sample is lowered to 4.5 or less.
4. The method of claim 1, wherein the pH of the sample is lowered to 4 or less.
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5. The method of claim 1, wherein the pH of the sample is lowered to 3 or less.
6. The method of claim 1, wherein the pH of the sample is lowered
25 to be in the range of from 2 to 5.5.
7. The method of claim 1, wherein the pH of the sample is lowered to be in the range of from 4.0 to 4.5.
- 30 8. The method of claim 1, wherein the pH of the sample is lowered to 5.5 or less by adding a buffer having a pH of less than 5.5.

9. The method of claim 8, wherein the buffer is a citrate, citrate phosphate, or acetate buffer.

5 10. The method of claim 1, wherein the concentration of 25-hydroxy-vitamin D is determined by immunoassay.

11. The method of claim 1, wherein the sample of blood or blood components is serum or plasma.

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12. The method of claim 1, wherein no precipitate is formed.

13. A method of claim 10, wherein a vitamin D tracer is used in the immunoassay.

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14. A method of claim 13, wherein the vitamin D tracer is ABEI conjugated to 25-hydroxy-vitamin D.

15. A method of claim 13, wherein the vitamin D tracer is ABEI
20 conjugated to a 22-carboxylic acid derivative of 25-hydroxy-vitamin D by a 2,2'-(Ethylenedioxy)diethylamine linker.

16. A method of claim 13, wherein the vitamin D tracer is ABEI
25 conjugated to a 22-carboxylic acid derivative of 25-hydroxy-vitamin D by a polyethylene glycol linker.

17. A method of claim 13, wherein the vitamin D tracer is ABEI
conjugated to a 22-carboxylic acid derivative of 25-hydroxy-vitamin D by a dimethyl adipimidate linker.

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18. A method of claim 13, wherein the vitamin D tracer is ABEI conjugated to a 22-carboxylic acid derivative of 25-hydroxy-vitamin D by a diamino cyclohexane linker.

5 19. A method of claim 13, wherein the vitamin D tracer is ABEI conjugated to a 22-carboxylic acid derivative of 25-hydroxy-vitamin D by a diamino C₃- to C₁₂- chain linker.

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